SUPPLEMENTARY MATERIAL

Study population, ascertainment of incident hypertension, risk factor assessment, and statistical analyses

Study population

The study population consisted of a representative sample of men living in the city of Kuopio and its surrounding rural communities in eastern Finland. Subjects were participants in the Kuopio Ischaemic Heart Disease (KIHD) risk factor study, a longitudinal population-based study designed to investigate risk factors for CVD, atherosclerosis and related outcomes.[1] Participants were 42-61 years of age during baseline examinations performed between March 1984 and December 1989. Of the 3433 potentially eligible and randomly selected men, 3235 were found to be eligible for the study. Of this, 2682 (82.9 %) volunteered to participate, 186 did not respond to the invitation and 367 declined to give informed consent. Men with a prevalent history of hypertension were excluded. The final cohort for the present analysis included 1,562 men with non-missing information on active serum calcium (iCa) and relevant covariates. The KIHD study complies with the Declaration of Helsinki and the Research Ethics Committee of the University of Eastern Finland approved the study, and each participant gave written informed consent.

Ascertainment of incident hypertension

Incident hypertension was defined as a physician diagnosis of hypertension, SBP $\geq$ 140 mm Hg and/or DBP $\geq$ 90 mm Hg, or use of anti-hypertensive medication as determined at re-examination rounds 4, 11, and 20 years after the baseline examinations. Cases were ascertained by record linkage to the national hospital discharge registry and to the Social Insurance Institution of Finland register for reimbursement of medicine expenses used for hypertension for the entire study period until the end of the follow-up. No losses to follow-up have been recorded in the KIHD study. Documents were cross-checked in detail by two physicians. The Independent Events Committee, masked to clinical data, performed classification of outcomes.

Measurement of risk factors

All biochemical assays were performed by a centralised laboratory in the University of Eastern Finland. Blood samples were taken between 08:00 and 10:00 hours. In addition to fasting, participants were instructed to abstain from drinking alcohol for at least 3 days and from smoking for at least 12 h prior to assessment. The serum samples were stored frozen at -80 °C for 0.2-2.5 years. The cholesterol

content of lipoprotein fractions and serum triglycerides were measured enzymatically (Boehringer Mannheim, Mannheim, Germany).[2] Fasting plasma glucose (FPG) was measured by the glucose dehydrogenase method (Merck, Darmstadt, Germany). Measurement of serum zinc concentrations was made from frozen serum samples stored at -20° C for 1-5 years prior to analyses, using the PerkinElmer 306 atomic absorption spectrophotometer (Norwalk, Connecticut, USA). Serum creatinine concentrations were measured by the colorimetric Jaffe method using a Konelab 20XT automatic analyser (Thermo Fisher Scientific, Espoo, Finland). Resting blood pressure was measured by a nurse on the first examination day with a random zero sphygmomanometer and the mean of all 6 measurements (3 supine, 1 standing, 2 sitting) was used as the final mean systolic and diastolic blood pressure.[3] Alcohol consumption was assessed using the Nordic Alcohol Consumption Inventory. Adulthood socioeconomic status (SES) was assessed as a combined measure of income, education, occupation, occupational prestige, material standard of living, and housing conditions.[4] History of diabetes was defined as having a clinical diagnosis of diabetes and regular treatment with diet, oral hypoglycaemic agents or insulin therapy, fasting plasma glucose $\geq$ 7.0 mmol/l, or according to self-reports. The energy expenditure of physical activity was assessed from a 12-month physical activity history modified from the Minnesota Leisure-Time Physical Activity Questionnaire.[5] This detailed quantitative questionnaire deals with the most common leisure-time physical activities of middle-aged Finnish men (conditioning physical activity, e.g. walking, skiing, bicycling, swimming, rowing, ball games, etc and nonconditioning physical activity, e.g. crafts, repairs, building, gardening, hunting, fishing, etc) and enables the assessment of all components of physical activity. For each activity performed, participants were asked to record the frequency, average duration, and intensity. Energy expenditure was measured for each physical activity by multiplying the metabolic index of activity (in metabolic equivalent*hour/week) by body weight in kilograms. Body mass index (BMI) was computed as the ratio of weight in kilograms to the square of height in metres.

Statistical analyses

Time-to-event analyses were conducted using Cox proportional hazards models[6] to examine the association of iCa with risk of incident hypertension after confirming assumptions of proportionality of hazards.[7] To characterize the shape of the association between iCa and risk of incident hypertension, hazard ratios (HRs) were calculated within quintiles of baseline iCa levels and plotted against mean iCa

levels within each quintile. Floating absolute risk (FAR) were used to calculate 95% confidence intervals for the log hazard ratio in each group, including the reference group, to allow for comparisons across the groups irrespective of the arbitrarily chosen reference category (bottom quintile).[8] This approach is utilized when more than two groups of the exposure are compared and it yields floated standard errors and floated confidence intervals. The FAR method incorporates sampling variation into the referent group and a confidence interval (CI) for the referent group is calculated. It also does not alter the estimated relative hazards. As the association showed an approximately linear shape, HRs were calculated per 1 standard deviation (SD) higher iCa levels. In subsidiary analysis, HRs were also calculated by quintiles defined according to the baseline distribution of plasma iCa levels. Hazard ratios were adjusted for age, BMI, systolic blood pressure, smoking status, history of diabetes mellitus, family history of hypertension, total cholesterol, high-density lipoprotein cholesterol, alcohol consumption, and physical activity. Further adjustment was made for estimated glomerular filtration rate (eGFR), as calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula,[9] SES, total energy intake, and serum zinc.

References

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